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CONTENTS

A SPECIFIC CHARACTER IN THE SUBFAMILY—TRUXALINAE (ORTHOPTERA—ACRIDIDAE)

M. G. Ramdas Menon and Baldev Parshad

Division of Entomology, Indian Agricultural Research Institute, New Delhi

INSECTS are known to produce sound in a number of ways with the help of specialised structures, known as stridulatory organs. Among the Acrididae the production of sound is either by the rubbing of the costal margin of the hind wing against the lower surface of the fore wing, or by the rubbing of the fore wings against a row of peg-like structures borne on the inner, lower side of the hind femora (Fig. 1 A). The latter method of production of sound appears to be restricted to a small family—Truxalinae—which according to Dirsh (1956) can easily be distinguished

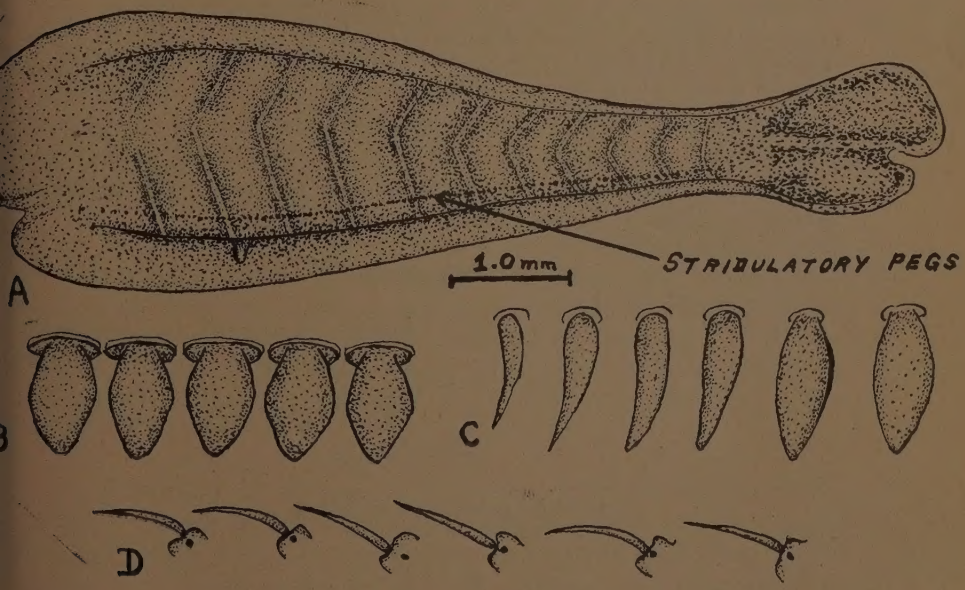

Fig. 1—A. Row of stridulatory pegs on the hind femur,
B. Pegs in the middle (magnified),
C. Pegs at the extremes (magnified),
D. Stridulatory hairs on the hind femur of *Acridella nasuta* Linn.

from the other subfamilies by the presence of these pegs. The shape and size of these pegs are very variable so much so that even those forming a row on a single femora are not alike. The pegs at the extremes are long, slender and pointed (Fig. 1 C) whereas those in the middle are short, stout and with blunt apices (Fig. 1 B). Another important feature about these stridulatory pegs is that their number remains constant for every species, thus affording a specific character. From amongst the species of Truxalinae represented in the National Pusa Insect Collection, the following five species were studied for this character and a constant number of these pegs was recorded for each of them. The number of pegs for each of these species is given below:

1. *Aswatthamanus cylindricus* Kirby .. 92 pegs
2. *Leva cruciata* Boliver .. .. .. 95 pegs
3. *L. indica* Boliver .. .. .. 101 pegs
4. *Aulacobothrus luteipes* Walker .. .. 108 pegs
5. *A. bolivari* Uvarow .. .. .. 118 pegs

In addition, a sixth species—*Acridella nasuta* Linn. which has been placed by Dirsh (1956) under the subfamily Acridinae has been found to possess a row of long slender bristle—like structures (Fig. 1 D) exactly at the same place where stridulatory pegs are observed in the other species and further their number is constant (124). The present authors are therefore of the view that the true systematic position of this species is under the subfamily Truxalinae and not under Acridinae.

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A CONTRIBUTION TOWARDS THE UNDERSTANDING OF THE FUNCTIONS OF THE PECTINES OF SCORPIONS

R. P. Sreenivasa-Reddy,

Department of Zoology, Sri Venkateswara University, Tirupati.

THE functional significance of the pectines of the scorpions has remained as one of the unsolved riddles of Zoology despite the fact that they are well known structures. Time and again various hypotheses have been advanced to interpret their functions (for a summary please see Millot and Vachon 1949; Cloudsley-Thompson 1955). These are neither adequate nor convincing for the following reasons. Three different types of sense organs have been reported to occur in the pectines (Schröder 1908). The occurrence of one of these has been questioned subsequently (Gossel, 1935). Hence in all probability the pectines have more than one function. In some of these hypotheses, the functions attributed to the pectines have no bearing on the histological structure of these organs (Blanchard 1853; Dufour 1856; Dumeril 1806; Gaskell 1902; Meckel 1829; Ubisch 1922). Moreover, the pectines show a wide range of variations in the number of teeth according to the species and sex. None of these hypotheses reckon with these as well as other significant features. Nor do they provide any explanation of the significance of these variations.

The specific and general aspects of this problem are being investigated in our laboratory. This communication summarizes the preliminary survey and deals mainly with certain correlations among the pectines, the morphology, ethology and the distribution of the scorpions. These correlations presented here also provide a theoretical basis for the major probable function of the pectines.

Materials

The data on the number of the pectinal teeth and other measurements for these studies were taken principally from Pocock (1900), Werner (1935), Berland (1948), Millot & Vachon (1949) and Vachon (1952). The morphology, ecology and zoogeography of the scorpions indicate that the variations in the number of pectinal teeth follow a basic pattern. Based on these data and information, graphs and maps were drawn, and the conclusions presented here are based on these.

The Correlations

A. *The chelae of the pedipalpi and the habitat of scorpions :* The chelae constitute an index of the habitat of the scorpions. As a general rule, the chactoid scorpions (Scorpionidae, Vejovidae, Chactidae, Diplocentridae and Bothriuridae) like *Heterometrus, Scorpio,* which are burrowing forms have wide chelae, well adapted for burrowing. The chelae of the buthoid scorpions (Buthidae) are comparatively narrow. Though they might be used for this purpose, they are admittedly not so well adapted. This would explain why the buthoid scorpions are usually found in shallow pits and other natural niches.

B. *Habitat and colouration :* These two groups differ from each other in their colouration also. The chactoid scorpions which usually live in cool humid burrows are relatively darker in colour than the buthoid forms.

C. *The length of the scorpions and the number of pectinal teeth :* Within each of these two groups, the buthoid and chactoid, there is a positive correlation between the maximum length of the scorpion and the number of pectinal teeth. The smallest scorpion is about 13 mm. (*Anoplobuthus parvus*) and the longest measures about 200 mm. (*Pandinus* sp.). The number of pectinal teeth ranges from a minimum number of about 3 (*Chaerilus margaritatus*) to a maximum of about 40 (*Parabuthus villosus* 42, *Buthus tamulus* 39, and *Androctonus australis* 38). In them the smaller species have fewer and the larger species higher number of teeth. Regression lines drawn for the total maximum length and the number of pectinal teeth have different coefficients and different slopes for these two groups.

D. *Habitat and the number of pectinal teeth :* On a broad basis the buthoid scorpions can be regarded as essentially xerophilic and the chactoid as hygrophilic. Within each group some members are found in other habitat also. Thus there are some buthoid scorpions which are less xerophilic and some chactoid forms which are less hygrophilic. As a general rule, the xerophilic scorpions have relatively greater number of teeth than the hygrophilic. For example, the members of the genera *Parabuthus, Buthus* and *Androctonus* which are xerophilic have the maximum number of pectinal teeth. Among the chactoid scorpions examples of typically hygrophilic forms are *Chaerilus margaritatus, Chaerilus granosus* (Sub. fam. Chaerilinae, Oriental Region) and *Belisarius xambeui* (Sub.

Fam. *Euscorpioninae*). The *Chaerilus* species referred to have 3-6 teeth, whereas *Belisarius* which lives in the caves of Pyrenees has only 4 teeth in each pecten.

The Buthidae in comparison with the remaining five families have a considerably greater number of pectinal teeth. Hence I propose to call the buthoid group as *Polydont* and the chactoid group as the *Oligodont* scorpions.

E. *Pectinal teeth and distribution of scorpions*: Among the polydont and oligodont scorpions, the species which have a relatively greater number of teeth are eurytopic in distribution. Those with relatively fewer teeth are stenotopic. For example, the genera *Buthus* and *Androctonus* not only have a comparatively greater number of teeth but also have a wide distribution. On the other hand *Charmus* (14-17 teeth) and *Stenochirus* (15 teeth) also belonging to Buthidae are stenotopic in distribution, confined to South India and Ceylon. Similarly among the chactoid scorpions the genus *Heterometrus* with relatively larger number of teeth has wide distribution (Indo-Malayan) and the genera *Chaerilus*, *Scorpiops*, *Iomachus* and *Calchas* which have fewer teeth have a narrow distribution.

To summarize, the polydont scorpions consisting of the Buthidae only, are poorly adapted for burrowing, are relatively less cryptozoic and live in comparatively drier habitats. On the contrary, the oligodont scorpions comprising of the remaining five families are essentially burrowing forms, live in comparatively humid places and are mostly cryptozoic. Again within the polydont and oligodont scorpions those which have relatively fewer teeth are confined to more humid places and those which have larger number of teeth live in arid regions. Thus there is a definite relationship between the number of pectinal teeth and the humidity of the habitat in which the scorpion lives.

The observations of Louis Fage, quoted by Berland (1948), on the scorpions of the genus *Grosphus* of Madagascar are of considerable interest in the present context. "The eastern part of the island has a humid tropical climate, while the western part has a warm and dry climate. In fact some regions of the western half are desert-like due to complete lack of rain. The distribution of the scorpions, particularly those of the genus *Grosphus* is determined by the climate. Of the different species of this genus *Grosphus* which inhabit the entire island, only two occur in the eastern half and the rest live in the western region. The species which

live in the dry region have highly developed pectines with greater number of pectinal teeth than those which inhabit the humid part". But Louis Fage asks " Y a-t-il là une relation avec respiration ? " (Is it associated with respiration ?). From the correlation presented here, it is clear that there is no direct relationship with respiration and that this is another striking instance of the interrelationship between the humidity of the habitat and the number of pectinal teeth.

F. *Pectines as adaptational structures* : Since the eurytopic species have greater number of teeth, it is quite probable that the pectines may be adaptational structures which enable the scorpions to colonize drier habitats.

G. *The function of the pectines* : The view that the pectines are primarily concerned with the humidity of the environment is not in discord with the structure of the sensilla basiconica which are considered as the most characteristic sensilla of the pecten. These sensilla basiconica are also of widespread occurrence in several orders of insects. While they are confined to the pectines of the scorpions they have a diffuse distribution on the body of the insects. These structures on the antennae of the grass-hopper have been demonstrated to be permeable to 0.5% aqueous solution of the acid fuchsin (Slifer 1954). In *Heterometrus fulvipes* the sensilla basiconica on the pectines are permeable to silver nitrate solution Hence the sensilla basiconica of the scorpions are likely to be hygroceptors.

H. *Pectines of the fossil scorpions from the Palaeozoic Era*: According to the classical view, the Palaeozoic scorpions were aquatic. The ventral view of the available fossils indicate the presence of the pectines in those scorpions. If the pectines can be regarded as adaptational structures for a terrestrial life, as revealed by these correlations as well as by their permeability, it is quite probable that the Palaeozoic scorpions also were terrestrial animals.

Detailed studies, both experimental and statistical, with reference to the correlations presented above, the functions of other sensilla and the nature of the habitat of the Palaeozoic scorpions are in progress and the results would be published in due course.

Summary

1. The chactoid scorpions are better adapted for burrowing and are more cryptozoic than the buthoid forms. The former which live in

cool and humid burrows are darker in colour than the members of the other group.

2. There is a positive correlation between the length of the scorpions and the number of pectinal teeth. The correlation co-efficients as well as the slopes of the regression lines of these two groups are different. The larger species have relatively greater number of teeth than the smaller.

3. The terms *Polydont* and *Oligodont* are proposed for the buthoid and the chactoid scorpions, respectively.

4. The polydont scorpions are poorly adapted for burrowing, are relatively less cryptozoic and live in comparatively drier habitats. The oligodont scorpions are well adapted for burrowing, live in humid places and lead a cryptozoic life. Thus a definite correlation exists between the humidity and the number of pectinal teeth.

5. The most characteristic sensilla of the pectines, the sensilla basiconica, are of widespread occurrence in the insects also. They are known to be permeable in the insects. These sensilla on the pectines of the scorpions are also permeable.

6. In the light of these correlations between the pectines and the habitat of scorpions, the pectines appear to be adaptational structures for a terrestrial life. If this is so, the Palaeozoic scorpions which were also, provided with pectines might have been terrestrial animals.

Acknowledgement

My sincere gratitude is due to Dr. Kandula Pampapathi Rao for the suggestion of this problem for investigation, guidance and help.

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STUDIES ON THE CONNECTING (CONDUCTING) TISSUE OF THE HEART OF GOAT

K. N. Bhargava,
Department of Anatomy, Medical College, Bhopal
AND
Ravi Prakash,
Department of Zoology, Hamidia College, Bhopal

[One plate]

MORE than a hundred years ago Purkinje (1845) observed, in the heart of sheep and a few other mammals, pale multinucleated fibres which now bear his name and are found to exist in the heart of all higher vertebrates. However, the functional significance of these fibres in the propagation of the impulse for the cardiac conduction was not fully understood till the researches of His (1893), Tawara (1906), Keith and Flack (1907) were known. His (1893) described a special muscle bundle at the atrioventricular junction of the heart of mammals for conveying the atrial stimulus of contraction to the ventricles. Tawara (1906) discovered the A.V. node and believed that this node controls the propagation of the contraction stimulus from atria to ventricles. Tawara also stated that the fibres of Purkinje, the bundle of His and the A.V. node described by him are all parts of a single atrioventricular conducting system. The discovery of a chain of impulse initiating and conducting structures was complete with the finding of the sinoatrial node by Keith and Flack (1907). Davies and Francis (1946), Kistin (1949), Walls (1947) and a few others, confirming the presence of the above mentioned structures, regarded them to be responsible for impulse initiation and conduction in the heart of birds and mammals. Prakash (1953, '54, '56, '57) observed all these structures not only in the heart of birds and mammals but also in the heart of some lower vertebrates. Against Davies and Francis (1946); Prakash (1954) maintained that the muscular conduction tissue of the hearts of birds and mammals has evolved from, and is a further specialisation of the tissue already existing for the purpose in the heart of lower vertebrates.

In contrast to the researches quoted above Todd (1932), Glomset and Glomset (1940), Glomset and Brige (1945 and '48) and Glomset and Cross (1952) did not support the myogenic theory of cardiac conduction

and questioned the conducting function of the nodal tissue and Purkinje fibres in the hearts of ungulates, dog and man. The views of Glomset and his co-workers have been explicitly summarised in the latest publication by Glomset and Cross (1952) where it has been stated that " Until it is proved that the nerve cells within the heart do not originate the cardiac impulse and that the impulse is not carried by the fibres of these cells to the individual units of the myocardium, it is folly and waste of mental energy to develop theory upon theory to explain impulse formation and conduction on the basis of a non-existant muscular conduction system. "

In the present investigation the heart of the goat has been studied with special reference to its conducting (connecting) tissue.

Material and Method

The sinoatrial node, the atrioventricular node, and the bundle of His with its two limbs were studied in the hearts of six young goats. All the hearts were fixed in Bouin's picroformol. The entire heart with the exception of the apex of each of the newly born and young goat was serially sectioned. In cases where the size of the heart was too big, large regional areas were sectioned serially. Sections were stained with haematoxylin and counter stained with eosin. The photomicrographs were taken from a Richerts Fibroscope.

Observations

The heart has four chambers *viz.*, two atria and two ventricles; there is no sinus venosus.

Sinoatrial node :

The sinoatrial node (Fig. 1) is present in the form of specialised tissue near the junction of the superior vena cava into the right atrium. This nodal tissue which is deeply embedded in the wall of the superior vena cava has interposed itself between the base of the superior vena cava and the cranial wall of the right atrium. The sinoatrial node is scattered in a way so as to surround the opening of the superior vena cava into the right atrium.

The node is characterised by the presence of Purkinje fibres and cells. Most of the cells of this node have prominent oval nuclei surrounded by a clear space of cytoplasm. A few cells have two nuclei instead of one. The fibres are long, slender and interwoven. In one of the series though no nodal artery could be located near the sinoatrial node, yet few arterioles

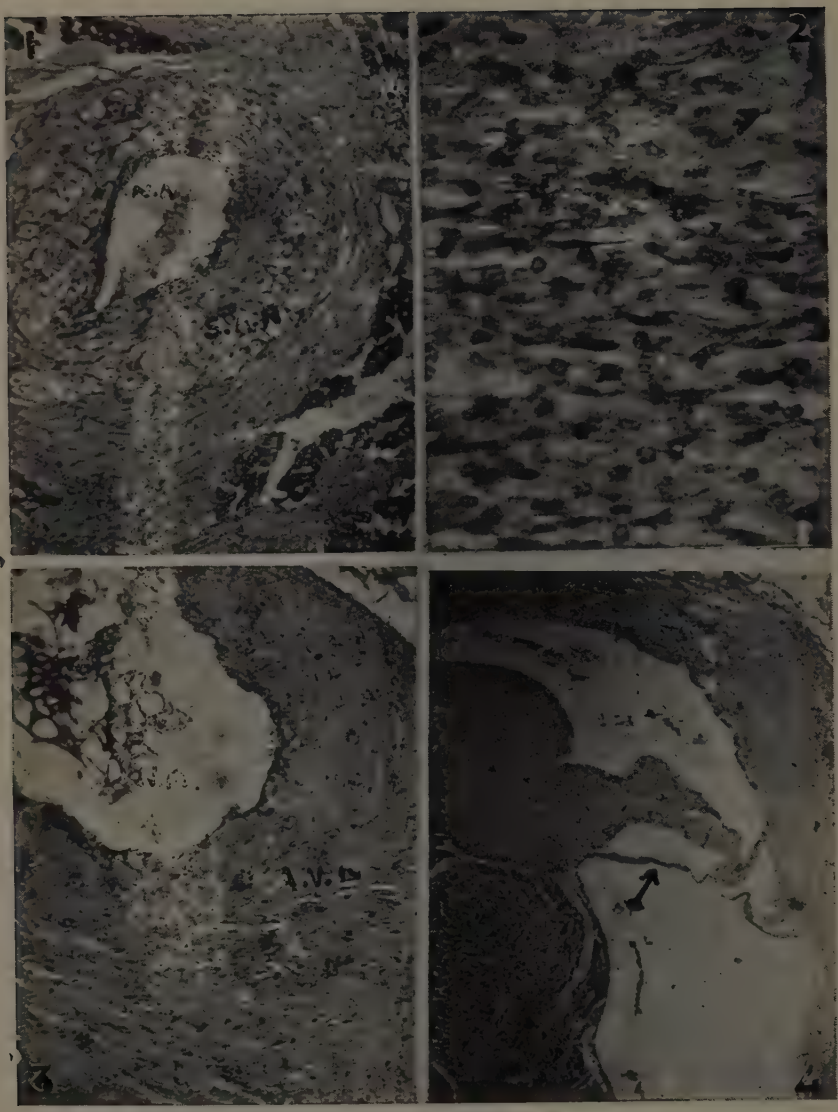

Fig. 1—Magnification X 250. Showing Sinoatrial node. SAN—Sinoatrial node. N.A. Nodal artery.

Fig. 2—Magnification X 250. Showing Interatrial Purkinge Fibres.

Fig. 3—Magnification X 250. Showing Atrioventricular node. A.V.N.—Atrioventricular node. N A.—Nodal artery.

Fig. 4—Magnification X 250. Arrow pointing to extension from the atrioventricular bundle in to the tricuspid valve.

and venous sinusoids were present. Even in the absence of a nodal artery the node could be easily identified because of its position and histologically specialised nature. A few nerve cells and nerve fibres were also present in the region of the node. Though the walls of the atria are totally devoid of any specialised fibres or cells, yet the Purkinje fibres are fairly distributed in the interatrial septum. (Fig. 2). These specialised fibres of the septum are continuous cranially with those of the sinoatrial node while caudally they extend into the atrioventricular node. Thus a continuous pathway of specialised fibres exists between the sinoatrial and atrioventricular nodes of the heart of goats through the specialised fibres in the interatrial septum.

Atrioventricular node :

The atrioventricular node (Figs. 3, 4) is situated in the caudal portion of the interatrial septum near the opening of the coronary sinus. The node could be recognized easily in almost every case at the cranical end of the interventricular septum. The rounded nodal mass is usually well demarcated from the the surronnding cardiac muscle.

The individual fibres of the A. V. node are thinner than those surrounding it and are much more interlaced than those of the sinoatrial node. Because of their interlaced nature these appear as cells having prominent nuclei with a surrounding cytoplasmic zone. The typical rounded and clearly demarcated atrioventricular node which has been observed near the base of the tricuspid valve has a nodal artery on either side of it.

Atrioventricular bundle :

The atrioventricular node is continued ventralwards directly into the atrioventricular bundle which is present in close association with the uppermost portion of the inter-ventricular septum (Fig. 4). The bundle of His and the atrioventricular node resemble the sinoatrial node in being formed of Purkinje fibres and cells. The bundle is enclosed within a definite layer of compactly arranged fibres, which gives it a definite shape and makes it distinct from the surrounding musculature of the interventricular septum. Moreover the component fibres of the bundle of His are smaller and much more loosely arranged than those forming the adjoining interventricular septum. Caudally the atrioventricular bundle bifurcates to form a right and a left limb of the bundle of His. These limbs of the bundle descend down along the respective sides of the interventricular septum. One of the most important features, perhaps hitherto not

recorded is the extension of the fibres of the atrioventricular bundle into the tricuspid valve as shown in figure 4.

Discussion

The controversy with regard to the presence of such an important structure as the sinoatrial node believed to be responsible for initiating the cardiac stimulus of contraction in vertebrate hearts, still exists. Keith and Flack (1907), Nonidez (1943), Davies and Francis (1946), Nomura (1952) and Copenhaver and Truex (1952) pointed out the presence of the sinoatrial node in several mammals while Todd (1932), Glomset and Glomset (1940) and Prakash (1954 a) denied the presence of this node in a few mammals. Prakash (1954 a) however stated that the sinoatrial node develops in mammals in relation with and consequent to the reduction of the sinus venosus. King (1954) pointed out that in serial sections of the rat's heart prepared by him, there was a definite sinoatrial node lying across the sulcus terminalis at the cranial extremity of the right atrium. Halpern (1955) also observed the presence of a horse-shoe shaped sinoatrial node lying around the right atriocaval junction in the hearts of the rat. Later on Prakash (1956 c) also reported the presence of S. A. node in the heart of the albino rat, where according to him the sinus venosus is absent. In confirmation of this view in the present study, it is observed that in the heart of the goat, a well defined and distinct S. A. node is present and a sinus venosus is absent.

Regarding the nature of the fibres that form the S. A. node, different views have been expressed by different workers. Davies (1942) considered the sinoatrial node to be a localised structure composed of a characteristic fibre type. Glomset (1942) and Glomset and Birge (1948) denied any specific structural characteristic for the fibres of sinoatrial node. Todd (1932) had earlier pointed out that the S. A. node is composed chiefly of Purkinje fibres, but according to Glomset and Glomset (1940) the slender size and less distinct striations of the fibres in the nodal region of the sulcus terminalis are merely alterations related to the amount of supporting tissue. Cophenhaver and Truex (1952) disagreed both with Todd and Glomset and stated that the fibre component of sinoatrial node does not resemble either that of the atrioventricular node or of the atrioventricular bundle in being totally devoid of Purkinje fibres. In the heart of the goat, however, it has been seen that the sinoatrial and the atrioventricular nodes present similar histological features in being

formed of Purkinje fibres. It is mostly because of the difference in the positions these two nodes occupy, that they have been indentified and named differently.

The present study supports Tawara (1906) who believed that the sinoatrial node, the atrioventricular node and the atrioventricular bundle, all form a continuum and are parts of a single atrioventricular conducting system involved in the conduction of the cardiac stimulus from atria to ventricles. Here it is also to be remembered that Tawara regarded the fibre component of the atrioventricular connecting tissue (S. A. Node, A. V. Node and A. V. Bundle etc.) to be of Purkinje type irrespective of its histological nature.

A noteworthy feature of the conducting system of the goat's heart is the presence of a special branch of Purkinje fibres from the bundle of His to the medial cusp of the tricuspid valve. Davies (1946) stated that correlated with functional requirements viz., to prevent regurgitation of blood into the right atrium, the right muscular valve of the bird's heart receives a special early branch from the right limb of the A. V. bundle. Prakash (1954) traced in the heart of a pair of human twins an extension of the right limb of the bundle of His towards the base of the medial cusp of the tricuspid valve. Drennan (1927) homologised the special branch of the right limb of the A. V. bundle of the bird's heart with corresponding elements in the mammalian heart. However, Prakash (1954 b) desired further study specially to decide whether Drennna was right or wrong to homologise the branch of the right limb of the mammalian bundle of His. On the basis of the present investigation in the heart of goats, where, in addition to the two limbs of the bundle of His, a separate special branch of Purkinje fibres to the medial cusp of the tricuspid valve is present, it is clear that the special branch of the A. V. bundle to the tricuspid valve of avian heart cannot be homologised with the right limb of the mammalian bundle of His.

Davis and Francis (1946), Robb and Turman (1948) and Copenhaver and Truex (1952) could not trace any connecting pathway of specialised fibres between sinoatrial and atrioventricular nodes. In this connection Copenhaver and Truex (1952) remarked "no one, as far as we are aware has described a Purkinje fibre pathway leading from the S. A. node to the atrioventricular node in any ungulate heart." In the heart of the goat Purkinje-like fibres are present in the interatrial septum and

they connect the sinoatrial node with the atrioventricular node. This makes the present study all the more interesting and important. The presence of such a continuity between the two nodes through Purkinje-like fibres of the atrial septum has revealed that in the heart of the goat, tracks of specialised fibres are present to conduct the contraction impulses from S. A. node to A. V. node.

Summary

The nature and disposition of the connecting tissue of the heart of goats has been examined and described. Nodal and Purkinje fibres are present. A sinoatrial node at the sinoatrial junction, an atrioventricular bundle at the cephalic end of the interventricular septum have been located and described. The special histological nature and position of these nodes and bundle has been taken as an evidence for regarding these structures responsible for impulse initiation, control and conduction in the heart of goats. The presence of special pathways formed of Purkinje fibres at the interatrial septum to connect the S.A. and A.V. nodes is a special and noteworthy feature. An extension of the fibres of the atrioventricular bundle into the tricuspid valve has been noted and its homology with similar extensions observed by other investigators in birds and mammals has been discussed. Against Glomset and his colleagues it has been pointed out that a special muscular conduction system exists in the heart of an ungulate.

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STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BIRDS

6. Variation in the Diameter of the Fibre of the *Pectoralis Major* and its relation to the Muscle Size and Mode of Flight

J. C. George and R. M. Naik

Laboratories of Comparative Anatomy and Animal Physiology, Department of Zoology, M. S. University of Baroda, Baroda.

VARIATION in the diameter of the muscle fibres in different muscles has been of considerable interest to myologists. Fibres of muscles differing in function have been studied with a view to correlate the size of the fibre with function. But, this approach has, however not met with much success. George and Jyoti (1955a) made some observations on the significant variation in the diameter of the muscle fibres of the *pectoralis major* muscle of a few birds and suggested the possibility of some relationship between the fibre diameter and the mode of flight. The present investigation is an attempt to explore further, into this most complex problem.

Material and Methods

All the birds used in the present work, except the fowl and partridge were either trapped or shot with an air-rifle. The pectoralis of each bird was removed a short while after the death of the animal and weighed immediately. Small pieces cut from it were used for obtaining the fresh frozen sections. The sections were mounted in 50% glycerol solution on microslides and under a magnification of eight hundred, the diameter of several hundreds of fibres from each preparation were measured using an ocular scale and a micrometer slide. In most of the birds the fibres in the superficial region of the muscle were somewhat bigger in diameter than those in the interior. So to obtain a correct mean value in such a muscle, measurement of the fibre diameter was done from areas selected at random from different depths of the muscle.

Results

The results obtained are presented in table no. 1, where the birds studied are grouped differently according to their mode of flight. It must however, be stated that grouping of birds as presented here is by no means

TABLE I

	Name of birds	Fibre diameter (μ) ± S.D.	Body wt. in Gms.	Muscle wt. in Gms.
A	Birds exhibiting shooting flight :			
	Indian Robin (*Saxicoloides fulicata*)	33.8± 8		1.0
	House Sparrow (*Passer domesticus*)	34.5±11		2.0
	Green Bee-eater (*Merops orientalis*)	38.0±10	15	1.5
	Red-vented Bulbul (*Molpastes hoemorrhous*)	38.3±10	33	2.3
	Mahratta Woodpecker (*Liopicus mahrattensis*)	40.2±6	37	2.5
	Blue-tailed Bee-eater (*Merops superciliosus*)	42.5±10	36	3.6
	White-breasted Kingfisher (*Halcyon smyrnensis*)	49.8±11	80	6.1
B	Birds exhibiting flapping flight :			
	Red-rumped Swallow (*Hirundo daurica*)	35.5±5		1.3
	Common Babbler (*Argya caudata*)	40.2±9	59	4.0
	Pied-crested Cuckoo (*Clamator jacobinus*)	37.0±6	80	4.2
	Common Myna (*Aeridotheres tristis*)	37.7±10	112	7.0
	Crow Pheasant (*Centropus sinensis*)	44.0±8	233	9.0
	Green Parakeet (*Psittacula krameri*)	36.6±7	118	10.25
	Blue Jay (*Coracia benghalensis*)	41.5±8		10.0
	Koel (*Eudynamis scolopaceus*)	41.9±9		12.0
	Common House Crow (*Corvus splendens*)	42.3±11		17.0
	Red-wattled Lapwing (*Sarcogrammus indicus*)	40.7±11		17.0
	Paddy Bird (*Ardeola grayi*)	42.7±10		18.0
	Cattle Egret (*Bubulcus ibis*)	43.2±10	347	21.0
	Owl	48.5±11		50.0
C	Birds exhibiting soaring flight :			
	Shikra (*Astur badius*)	41.2±13	148	11.3
	Common Pariah Kite (*Milvus migrans*)	46.6±9		39.0
	White-backed Vulture (*Pseudogyps bengalensis*)	66.2±13	2509	341.0
D	Nonflying birds :			
	Partridge (*Francolinus pondicerianus*)	60.8±15		12.0
	Fowl (*Gallus domesticus*)	64.0±16		47.0

based on the assumption that birds within a particular group indulge only in the one particular mode of flight mentioned, but rather it should be understood that, that mode of flight attributed to the group is the most usual and characteristic, while those birds might resort to other type or types of flight occasionally for short durations. For example, many of the birds grouped under B, often exhibit shooting type of flight ; Shikra (a

hawk), though listed as soarer (group C), while moving from tree to tree or while flying at low altitudes does flap its wings constantly with ease. The weight of the bird wherever recorded is mentioned so as to give some idea about the size of the animal. The values for the diameter of the fibres is the mean of several hundreds of fibres measured. Wherever, more than one specimen of one type were used, the mean of the values obtained from different specimens is given. However, in no case individual variations were found to be sufficiently significant for special consideration. It appears that the mean fibre diameter of the pectoralis is fairly constant for a bird provided it is fully grown, normal and active.

In birds belonging to any single group, the diameter of the fibres increases with the weight of the muscle. Figure 1 is a graphical representation of the relation between the fibre diameter and muscle weight. It can be observed that all the birds in group A fall along the line *a*, the formula for the regression line is $y = 31.2551 + 3.1023x$ ($r = 0.96$). Similarly, the birds of the group B fall along the line *b*, where $y = 37.6203 + 0.2618x$ ($r = 0.58$). It will be observed that the slope of the line *a* is much steeper than that of the line *b*. Since the number of birds observed in group C and D are fewer in number, no accurate relationship for line *c* and *d* can be derived. But, it appears that the birds of the group C do not differ significantly from the group B as far as the relation between the diameter of the fibres and muscle weight is concerned.

Discussion

The size of a cell in any tissue is normally considered to be a fixed entity. In a bigger animal, it does not become bigger, though cells may increase in number to form a bigger structure. An individual muscle fibre though multinucleated, since is derived from a single myoblast, may be regarded as a cell. In that case, the present work shows that the muscular tissue is an exception to the above stated general rule. It appears that in homologous muscles with identical functions, diameter of the fibres varies with the size of the muscle.

With increase in fibres diameter, the distance between the centre of the cell and the blood capillaries lining its border increases. Hill (1956) pointed out that, " In objects of similar shape, the time taken for diffusion to operate varies directly as a square of the linear size". The rate of supply of dissolved substances to the interior of the cell decreases with the

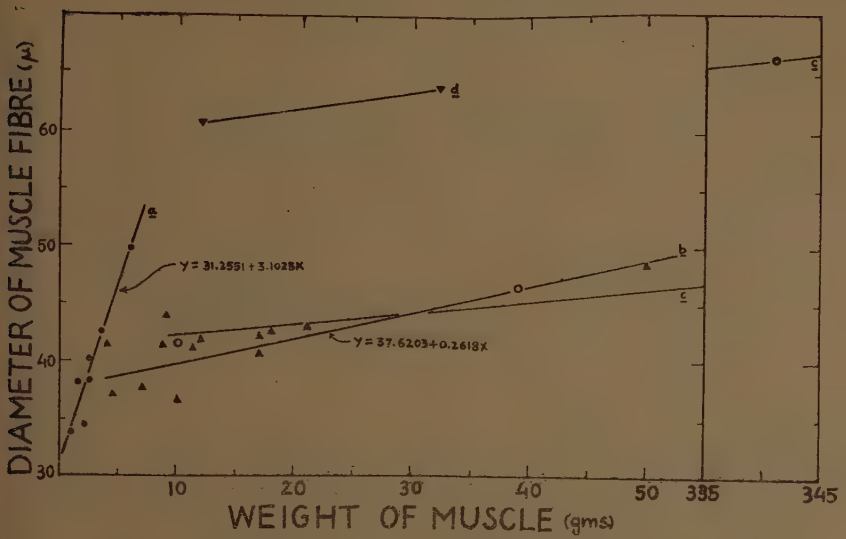

FIG. 1 Relation between the fibre diameter and weight of the *pectoralis major* in birds—line *a* for birds (●) exhibiting shooting type of flight, line *b* for birds (▲) exhibiting flapping type of flight, line *c* for birds (○) exhibiting soaring type of flight, line *d* for non-flying birds (▼).

increase in the fibres diameter. Under these circumstances, unless the inherent requirement of oxygen in bigger muscles is less, the muscle cannot function effectively.

Again, according to Hill (1956), "The total power output of similar animals increases about as the square of their linear size, so the maximum oxygen requirement per gram weight diminishes inversely as the linear size". This deduction derived from principles of thermodynamics, for an entire animal, can as well apply to an entire muscle. Larger muscle should have higher total power output and consequently less oxygen requirement per gram weight. Moreover, comparing the diameter of fibres with the muscle weight, has several advantages. The size of the animal may be expressed in terms of body weight or surface area. Table no. 1 shows that the diameter of the muscle fibres presents a closer relationship with muscle weight than with the body weight. The surface area is a better index for size, but an accurate direct measurement of it is by no means simple. The square of the animal's length is often taken as an index of the surface area, but it is no longer comparable in animals with different shapes, as in birds, where great modifications in the length of the neck and appendages are a common feature.

In groups B and C, the relation between the weight of the anima and fibre diameter essentially remains the same. Birds exhibiting shooting type of flight form altogether a different line with a very steep slope, thus differing sharply from the birds grouped under B and C. The fibre diameter in the pectoralis of these birds (group A) is comparatively bigger. Since, with the increase in the size of the muscle, the increase in the diameter of the fibres is considerable, the decrease in the rate of diffusion of dissolved substances should be appreciable. Possibly, the mode of flight of these birds, in which every quick succession of wing flaps is followed by a short period of rest, might be the underlying reason. It is quite significant that this mode of flight is confined only to smaller birds.

Average fibre diameter of birds in group D is highest among the birds studied and the limitations it enforces on tissue respiration is well correlated with the non-flying habit of these birds. The babbler and crow pheasant (included in group B) with broad and short wings are poor fliers, in that, their wing beats are fast but of very short duration and it is not surprising that they have fibres with greater diameter when compared to other birds in group B.

The present investigation is in no way conclusive and it is necessary that many more birds, especially those of larger size should be examined before the functional differences in flying birds as revealed by this work is firmly established. Nevertheless, the present observations have opened up some new avenues in the study of the functional anatomy of muscles.

Summary

1. In birds with similar flying habits, the mean diameter of the fibres in the *pectoralis major* muscle varies with the size of the muscle.

2. The rate of increase in the diameter of the fibres per gram weight of the muscle differs in birds with different flying habits.

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STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BIRDS

7. The Structure of the *Pectoralis Major* Muscle of the Pigeon in Disuse Atrophy

J. C. George and R. M. Naik

Laboratories of Comparative Anatomy and Animal Physiology, Department of Zoology, M. S. University of Baroda, Baroda

[2 plates]

THE *pectoralis major* muscle of the pigeon, as a mixed type of muscle, has drawn the attention of several workers. Histological and physiological studies on this muscle have revealed several fundamental differences between the two types of fibres, *viz.* red and white fibres, (Denny-Brown, '29 ; George and Naik, '57, '58a, '58b and '58c ; George and Scaria, '58a, '58b and George, Scaria and Nair, '58). The reactions of these fibres in muscular atrophy is therefore of special interest.

According to Adams, Denny-Brown and Pearson ('54), the differences between ' the pigmented and pale muscle fibres ' in a mixed type of muscle are no more evident at the end of two months after denervation. All muscle fibres contain some fat granules and pigment as in the foetus. Knoll and Hauer (1892) showed that the great differences between the " pale large " and the " dark small " fibres vanish by thirty fifth day after denervation. The present work deals with the structural changes in the two types of fibres in disuse atrophy.

Materials and Methods

Of the two male pigeons utilised in the present work one (pigeon no. 1) was kept along with a female in a rectangular cage (18″ × 24″ × 18″) for about two years. Due to the small size of the cage, the bird could only move about on the floor and flight was impossible. The only possible movement of the wings was that for the adjustment of feathers and a few flaps in the morning and occasionally at other times of the day, apparently for ' warming up '. During the entire period of captivity, they were fed regularly with their normal diet, were normal in every respect and they even reared up several broods of young ones. At the end of the two years

of captivity, the male was sacrificed and the structure of its pectoralis studied from sections (t.s. as well as l.s.) of fresh frozen muscle pieces and of those fixed in Zenker-Formol and embeded in paraffin. Fresh frozen sections were studied unstained, whereas the paraffin sections were stained with Ehrlich's Haematoxylin and Van Gieson's Picro-Fuschin.

Another male pigeon (no. 2) had its right shoulder joint fixed in a plaster cast, which restricted to very great extent the movement of the humerus at that joint, though a few extremely slight movements were still possible. Moreover, since the plaster cast was put all round the joint in the form of a broad circular ring, the wing could not be flexed completely, with the result that, the upper arm remained in a slightly extended position. The pigeon was kept in this manner in a large cage along with some other normal pigeons, on a normal diet. In the beginning, it had some difficulties in balancing itself for a few days, but soon got accustomed to it. It was observed to flap the left wing, with ease, quite often for balancing, while running about in the cage and for movements of courtship, offence and defence. At the end of three months the animal was decapitated and the *pectoralis major* of both sides were cut out. The left pectoralis was used as control and the muscle pieces cut out from it were studied in the same manner as those from the right pectoralis. For immediate inspection fresh frozen sections were used, whereas frozen sections of muscle pieces, from different regions of the muscle, fixed in Baker's Calcium-Formol and embedded in gelatin, were stained with Ehrlich's Haematoxylin and Eosin, Sudan Black B and Eosin and Oil Red O and Haematoxylin.

Results

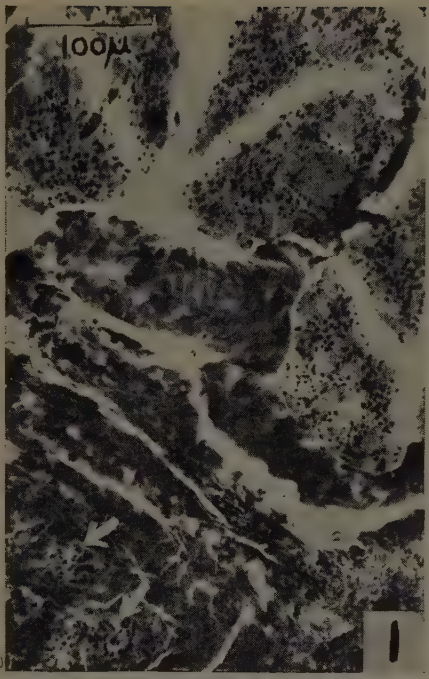
In the normal pigeon pectoralis, the red and white fibres, due to their sharp and clear cut differences and definite pattern of arrangement, could be easily distinguished in stained or unstained histological preparations of fixed or unfixed muscle. Throughout the present work the sections of the normal pectoralis were used as an useful guide.

PLATE 1

Explanation of Figures

Structure of the *pectoralis major* muscle of the pigeon no. 1 in disuse atrophy.

Figures 1-5. Microphotographs of sections (1, 2 and 4—T. S.; 3 and 5—L. S.) stained with Ehrlich's Haematoxylin and Van Gieson's Picro-Fuschin. (Two arrows in figure 1 indicate clumps of nuclei of degenerated muscle fibres, surrounded by dense aggregate of connective tissue.)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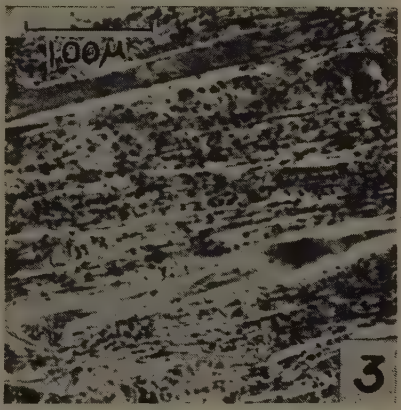
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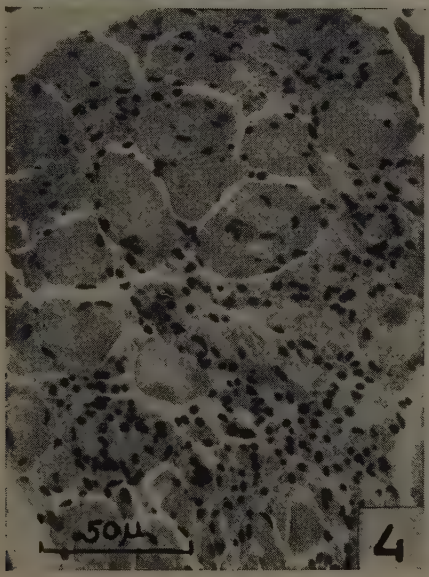
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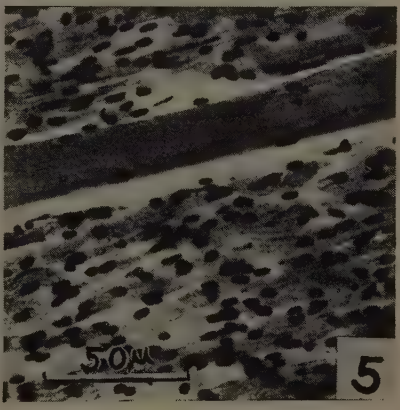
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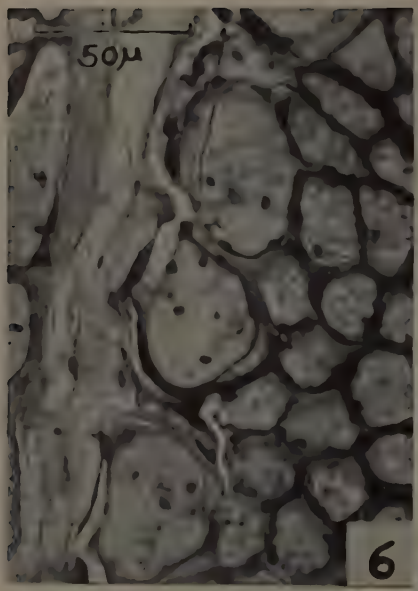
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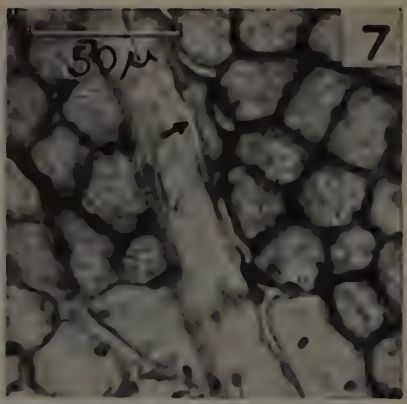
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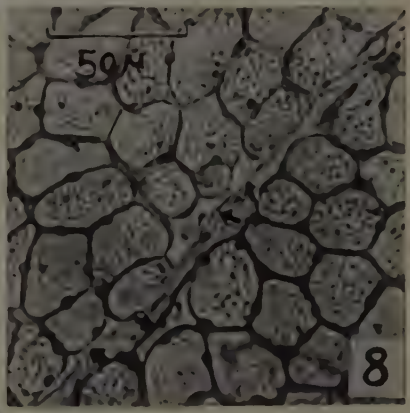
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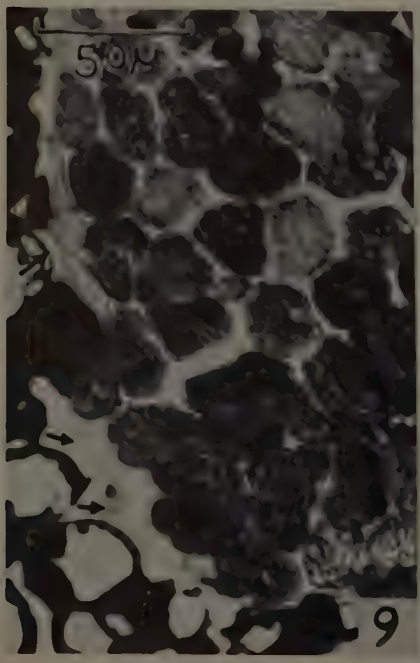
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In pigeon no. 1 the pectoralis muscle of each side was extremely reduced in size and paler in colour and likewise, in fresh frozen sections, the narrow fibres appeared paler and less granulated compared to those in normal pigeons, but the granulation in the white fibres, just as in normal ones was negligible or nil.

Figures 1-5 (plate 1) present the structure of the pectoralis of pigeon no. 1, showing the signs of atrophy. Both types of fibres were affected. All the red fibres were extremely reduced in diameter. Among the white fibres few fibres here and there were reduced but little, while majority of them showed varying extents or reduction in size, with the result that in any preparation white fibres of all sizes from broadest to narrowest could be found. The striations were clear in all the fibres however reduced and none of the fibres showed any sign of fragmentation.

The nuclei stood out prominently in all the preparation and were oval or round in shape (figure 5). On observing under oil-immersion the nuclei appeared granulated and with prominent nucleous. Some elongated nuclei, beaded at two ends with an intervening narrow portion gave the indication of an amitotic nuclear division. But from the present work it is difficult to say whether there was any definite increase in the number of nuclei or not. The red fibres appeared as tubes filled with nuclei. In the white fibres some nuclei were seen away from the periphery, while others were at their normal position (figures 4 and 5).

A definite increase in the amount of connective tissue in the perimysium was seen. In certain regions thick bands of connective tissue extended in between the fasciculi (figures 1 and 2). In figure 1, towards the left at the bottom, two large clumps of nuclei resembling those of

PLATE 2

Explanation of Figures

Structure of the right *pectoralis major* of the pigeon no. 2 in disuse atrophy.

6. T. s. passing through the portion of the muscle proximal to the ventral face. Ehrlich Haematoxylin—Eosin.

7. T. s. passing through the deeper region—at about mid-depth of the muscle. Ehrlich's Haematoxylin—Eosin. (Of the four white fibres seen in the figure, the one—in advanced state of atrophy—is indicated by arrow.)

8. T. s. passing through the portion of the muscle, proximal to the dorsal face of the muscle. Ehrlich's Haematoxylin—Oil Red O. (White fibres are indicated by arrows.)

9. T. s. passing through the deeper region of the muscle. Sudan Black B—Eosin. (White fibres are indicated by arrows.)

10. L. s. through the deeper region of the muscle showing fat deposition around branches of a blood vessel. Haematoxylin—Eosin.

muscle fibres are seen in the midst of a thick aggregate of connective tissue, suggesting the possible replacement of muscular tissue by connective tissue. Large aggregates of fat cells, as are usually associated with the degenerative changes in the fibres of denervated muscle, were however not observed in the muscle.

In pigeon no. 2, the right pectoralis was somewhat more reduced in size than the left one, which was used as control. Histological observations showed that throughout the latter muscle the fibres were normal in every respect. On the other hand, white fibres in the right pectoralis showed signs of atrophy which at different depths of the muscle varied in magnitude. Figure 6 (plate 2) shows portions of two fasciculi from the region proximal to the ventral face of the muscle, where the effect of atrophy was very slight. Both types of fibres appeared similar to those in the control except that in many of the broad fibres some of the nuclei had migrated away from the periphery.

Figure 7 represents a typical portion in the deeper layer at about the mid-depth of the muscle. All broad fibres show varying reduction in size. Three broad fibres at the bottom of the figures are smaller in diameter compared to those in figure 6, while at the upper part of the figure a white fibre can be seen very much reduced in size and flattened out against the border of the fasciculus. Migration of nuclei away from the periphery of white fibres was a prominent feature in this region. Size of the red fibres was unaffected and except for an occasional fibre, most of them had nuclei in the normal position.

Figure 8 presents the picture of a still deeper layer of the muscle. Here all the white fibres show great reduction in size. Those on the periphery of the fasciculi (indicated by arrows) can be seen in the form of flattened out ribbons, while those in the middle of the fasciculi (not shown in the figure) were not flattened but instead, deeply indented, angular and much reduced. Still the red fibres showed almost no sign of atrophy—they were still normal in size and in the position of their nuclei. In this region as well as another still proximal to the dorsal face of the muscle, at several places white fibres had disappeared altogether, leaving little or no remnant of the muscle fibre with the result that the borders of the fasciculi were broken up and gaps formed in the regions where the white fibres degenerated. Whether the fibres were replaced by connective tissue or not, however, could not be ascertained.

Figure 6 clearly shows the differences in the structural details of the two types of fibres as in the normal muscle. In the red narrow fibres, the Cohnheim's areas were very prominent and irregular, whereas in the white fibres they were less conspicuous and formed a regular pattern. Almost the same differences in the structure of the two types of fibres remained at any depth of the muscle even where the white fibres were in an advanced state of atrophy.

Like red fibres in general, those of the pigeon breast muscle have greater affinity for Sudan dyes, unlike the white fibres. In the present investigation, the red fibres towards the deeper layers of the right pectoralis of pigeon no. 2 showed more fatty inclusions, the lipoid globules being more numerous, than those in the superficial layers or in the normal muscle. Figure 8 shows lipoid inclusions in the red fibres prominently, whereas in the white fibres, even in the advanced state of degeneration no such inclusions are seen.

Figure 9, which also is of the deeper layer of the muscle, shows the relative affinity of the two types of fibres distinctly. All along the bottom and the left margin of the figure aggregates of fat cells are seen. Between intensely stained fat cells and the darkly stained red fibres are three flattened white fibrers (indicated by arrows) with almost no deposition of Sudan Black. It should however be noted, that the higher deposition of fat is mainly in the ' intercolumnar ' cytoplasm of the red fibres and that this deposition should not be confused with the replacement of the muscular tissue by fat cells, as it usually happens in certain degenerative processes of the muscular tissue. No where in the present case the replacement of muscle fibres by fat cells was observed. No doubt, in the deeper layers of the muscle, large aggregates of fat cells were present in the perimysium (figure 9) and isolated fat cells were present in the endomysium (visible near the upper border of figure 9) ; wherever the large aggregates of fat cells were present they were mainly in the vicinity of large blood vessels. Figure 10 shows such fat deposition around several branches of a blood vessel.

Discussion

Knoll and Houer (1892) reported that the grest differences between the ' pale large and dark small ' fibres of pigeon after denervation no longer existed. Our observations show that in disuse atrophy the main differences, namely that of colour and lipoid inclusions are well maintained.

In pigeon no. 1, where the animal was caged, in a limited space for a long period, inactivity produced atrophy in both the types of fibres in the same manner. Since the white fibres were originally broader than the narrow ones at all stages in the process of atrophy they should be bigger in diameter than the red fibres which too should reduce in size in a like manner.

On the other hand in pigeon no. 2 where the activity of the pectoralis was limited due to control of movements at the shoulder joint, changes due to atrophy were much more profound in the white fibres than in the red ones, which seem to be less suceptible to atrophy. The white fibres proximal to the ventral face of the muscle were comparatively less affected than those in the deeper portion of the muscle. This suggests that under the present experimental conditions, the activity of the muscle in the deeper region has been more restricted than in the superficial part (proximal to the ventral face) of the muscle. This experiment, doubtless, reveals that the white fibres are more suceptible to atrophy than the red ones, but however no definite reason for it can be given because of the lack of data from similar experiments under altered conditions. Nevertheless, the observations of Langley made as far back as 1917 on the muscles of the denervated cat limb are worthy of special consideration. He showed that in the denervated cat limb, the loss in weight of the soleus which is a red muscle was considerably less than that of the white muscles and that during regeneration the former recovered from atrophy faster manifesting less fibrilation, than the white muscles. The mode of action of some factors involved in the conditions of the present experiment also need to be checked. For instance the movement at the shoulder joint was not totally eliminated and since the plaster cast rendered the upper arm to be in a slightly abducted position, the muscle was obliged to remain slightly stretched and not in its usual resting position. The present findings nevertheless clearly indicate the possibility of existence of some basic differences, still unknown, possibly in the physico-chemical properties of the two types of fibres in the muscle.

Summary

1. Observations on the effect of disuse atrophy on the *pectoralis major* muscle of the pigeon are reported.

2. The *pectoralis major* of a pigeon captivated in a small cage for two years, showed the effect of atrophy on both the red, narrow and the

white, broad types of fibres. Another pigeon in which muscular atrophy was produced by fixing a plaster cast on the shoulder joint, showed at the end of three months high degree of atrophy in the white broad fibres whereas the red narrow ones remained unaffected.

Acknowledgment

One of us (R.M.N.) is indebted to the Ministry of Education, Govt. of India, for the award of a Senior Research Scholarship.

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STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BIRDS

8. Adenosinetriphosphatase Activity and Sulphydryl Groups in The Pigeon Breast Muscle

J. C. George and S. D. Pishawikar

Laboratories of Animal Physiology and Histochemistry, Department of Zoology, M. S. University of Baroda, Baroda.

THE pectoralis of the pigeon as a mixed muscle is of considerable interest to muscle physiologists. George and Naik (1958a, b, 1959) who made a study of this muscle showed that the red narrow fibres are loaded with fat and mitochondria whereas in the broad white fibres these inclusions are considerably less and that the latter contains about five times more glycogen than the former. Higher lipase and dehydrogenase activity in the red narrow fibres has been reported by George and Scaria (1958 a, b). Alkaline phosphatase at pH 9.2 higher in the narrow fibres is considerably less in the broad fibres and is confined to the periphery only (George *et al*, 1958). In the light of the above observations it was thought necessary to investigate the metabolism of high energy phosphates in the above two types of fibres in the pigeon pectoralis. In this report histochemical localisation of ATPase and - SH groups in the pigeon pectoralis is presented.

Material and Methods

Strips of muscle were cut from a decapitated animal after complete draining of blood and were then rapidly frozen on the stage of the freezing microtome. Transverse sections were cut at 40μ to 80μ and transferred to clean dry slides. The sections were then dried in a vacuum desiccator over calcium chloride at room temperature, fixed in 10% neutral formalin at 4°C for one hour and after washing in tap water for one hour and rinsing in distilled water, were transferred to incubation mixtures. The method employed was that of Pearse and Reis (Pearse, 1954) using ATP as substrate. The sections were incubated for two hours at 37°C at a pH of 7.4 and the rest of the procedure was as followed by the above authors. Sections incubated in substrate blank medium, those incubated in the mixtures containing glycerophosphate as substrate at pH 7.4 and

9.2 and sections kept in boiling water for 10 minutes prior to transferring in the incubation mixtures, were used as controls. Sections with zero hours incubation were also used as control. For the demonstration of free - SH groups fresh frozen sections were stained with a drop of 2% sodium nitroprusside solution saturated with ammonium sulphate and containing concentrated ammonium hydroxide (Glick, 1949). Some of the sections were immersed in 10% trichloracetic acid (TCA) for 15 minutes and washed in water prior to staining with nitroprusside so as to demonstrate the bound - SH groups.

Results and Discussion

Figure 1 shows the localization of ATPase in the two types of fibres. The enzyme activity is considerably higher in the broad white fibres than in the red narrow ones. For the demonstration of free and bound - SH groups the red colour produced was found to be more in the broad white fibres than in the red narrow one.

Waschstein and Meisel (1956) while demonstrating ATPase in muscle at pH 7.2 reported that some of the fibres were stained darker than the others, while Nechmias and Padykula (1958) got a uniform staining in mixed muscle of rat and suggested that ATPase demonstrated by Wachstein and Meisel was mitochondrial ATPase and that the difference in staining obtained was due to the difference in the mitochondrial

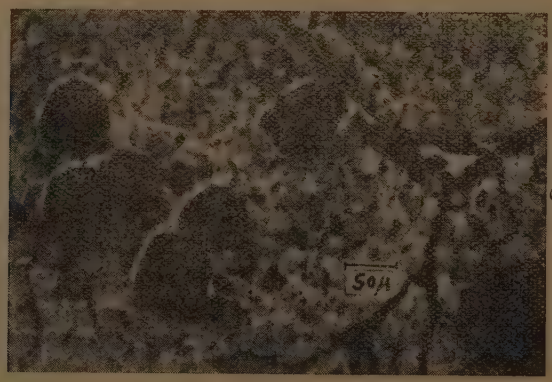

Fig. 1—T. S. of the *pectoralis major* of pigeon showing the relative concentration of ATPase in the two types of fibres. The broad fibres which appear dark indicate a considerably higher concentration of the enzyme in them than in the narrow ones which are comparatively much lighter.

content of the different fibres. In pigeon breast muscle the mitochondrial content of the broad white fibres is nigligible when compared with that of the narrow red ones in which mitochondria are abundant and the ATPase stained is much more in the broad white fibres than in the red narrow ones as is very clearly seen from the figure. Since the broad fibres contain few mitochondria, in the present study the ATPase demonstrated is not the mitochondrial ATPase but probably myosin or actomyosin ATPase. Again it is not possible to say whether it is only myosin ATPase because Actomyosin as such is also found in the muscle extracts and that the Actomoysin ATPase is known to have an optimum pH of 7.4. It has been mentioned earlier that the broad white fibres have been shown to contain only negligible concentrations of oxidative enzymes and alkaline phosphatase unlike in the narrow fibres. The higher concentration of the - SH groups in the broad fibres therefore is probably due to the higher concentration of ATPase and other phosphatases (*viz.* acid phosphatase, pH. 5. unpublished).

One of us (S.D.P.) is indebted to the Government of India for the award of a senior Research Scholarship which made this work possible.

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SEX CHANGES IN A WOOD-BORING MOLLUSC, *BANKIA (LILIOBANKIA) CAMPANELLATA*

P. N. Ganapati and R. Nagabhushanam

Department of Zoology, Andhra University, Waltair

THE wide variation in the expression of sexuality among the lamellibranchs is also found among teredines. Sigerfoos (1908) found many hermaphrodites among the young individuals of *Bankia gouldi* and suspected that protandry is normal in this species. Coe (1941) observed that in *Bankia setacea* functional hermaphroditism occurs only occasionally in the primary male phase; the subsequent sexual phases are clearly differentiated, often with a resting stage intervening between the two sexual phases. The sexual phases are of the alternative type in that any sexual phase, after the first, may be followed by either a male or female phase if the length of life suffices (Coe, 1941). Nair (1956) recorded protandry in *Bankia indica*, nearly all females passing through a preliminary functional male phase before reaching the female stage. It is also suggested that the female after a functional spawning phase can revert to the male phase and produce spermatozoa. The sexual sequence in other species of *Bankia* are not known at present.

During the course of our investigation on the biology of *Bankia* (*Liliobankia*) *campanellata* a systematic study of the sequence of the sexual phases was also made. The results are briefly summarized and compared with previous reports on the sexuality of other species of *Bankia*.

Material and Methods

The material for the study was collected by immersing standard test panels (6″ × 6″ × 1″) of deal-wood immersed in Visakhapatnam harbour. In two months sufficient material was obtained for the analysis.

To study the sequence of sexual phases in *Bankia campanellata*, the sexual condition of three samples consisting of 200 specimens were examined and grouped according to their sizes. The length of the burrow was taken as the length of the animal and the gonads teased and examined microscopically. The gonads of about 50 forms of different size groups were fixed in Bouin's fluid and later sectioned in iron Haematoxylin for detailed histological study.

Results

Table I contains the results of examination of gonads of different size groups. In the first size group, of the 35 specimens all showed ambisexual gonads. In the second size group (between 20 and 50 mm. in length) out of 84 forms there were 11 forms which had no ovocytes on the follicle walls, the entire lumen of the follicles of the gonad being filled with spermatozoa. These represent the 'true males' of Coe (1933). In this size group there were 65 ambisexual males in which the gonads showed a highly variable proportion of spermatogenic and ovogenic cells (Photomicrograph 1). Serial sections showed a cortical layer of small inactive ovocytes and the lumen of the follicle containing spermatozoa. There were only two hermaphrodites where both the types of sexual products ripened simultaneously. 6 specimens were females and as Coe (1933) says such females make their appearance by the omission of the initial male phase. Usually *B. campanellata* attains this size of about 50 mm. in a month.

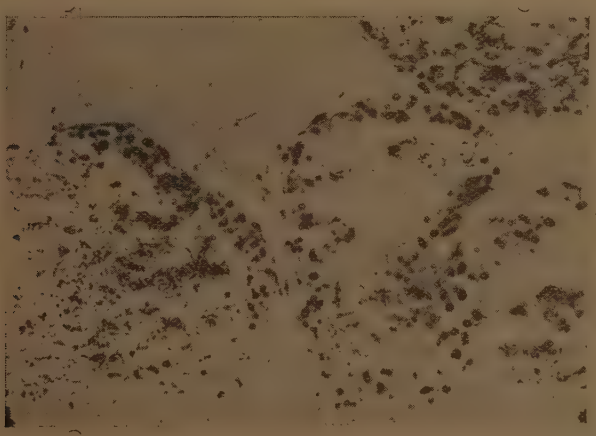

Fig. 1—Photomicrograph of the section of the ambisexual male gonad showing cortical layer of developing ovocytes with spermatozoa in centre.

In the third size group there were 42 females out of 61 forms examined. Evidently these are the forms which have changed their sex from initial ambisexual male phase. In the last size group, out of 20 forms examined, there are 14 females and 6 males and no hermaphrodites or ambisexual males.

TABLE I

Showing the Sexual condition of the specimens examined

Length in mm.	True Males	Ambisexual Males	Hermaphrodites	Females	Total
1-20	—	35	—	—	35
20-50	11	65	2	6	84
50-100	3	13	3	42	61
100 and above	6	—	—	14	20

From the above account it is clear that *B. campanellata* just like other species of *Bankia* is essentially protandric, the majority of individuals passing through a functional male phase before reaching the female condition. Fully adult individuals measuring 100 mm. and above in length, have the appearance of either males or females with no indication of ambisexuality.

Summary

Bankia (Liliobankia) campanellata is essentially protandric, the majority of individuals passing through a preliminary functional male phase before reaching the female condition. It exhibits consecutive sexuality as *B. setacea*.

Acknowledgement

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A QUANTITATIVE STUDY OF THE PROTEIN-BOUND AMINO ACIDS IN THE YOLK AND EMBRYO DURING THE DEVELOPMENT OF *ORYZIAS MELASTIGMA* McCLELLAND

VIJAYAM SRIRAMULU

Marine Biological Station, Annamalai University, Portonovo

A recent study by the author (Sriramulu, 1958, 1959) on the qualitative and quantitative changes in the protein bound amino acids during the development of *Oryzias melastigma*, has shown that the concentration of the amino acids increases during gastrulation and decreases gradually and at hatching shows a marked drop.

A problem of special interest in the development of yolky eggs relates to the utilisation of the amino acids of the yolk by the embryo. It would be interesting to compare the changes in the patterns of distribution of amino acids in the yolk and embryo during development. Practically no investigation seems to have been carried out so far on this aspect. Recently Rupe *et al.* (1955) studied the distribution of fourteen amino acids on the white, yolk and embryo during the incubation of hen's egg and found that for the first 250 hours of development the 'white' supplies the major portion of the amino acids needed by the embryo. The present study relates to the quantitative changes in the protein-bound amino acids in the yolk and embryo during the embryogenesis of *Oryzias melastigma*.

Material and Methods

The eggs of *Oryzias melastigma*, collected from local ponds and laboratory aquaria, were washed well with filtered water to remove any adhering particles of debris and then allowed to develop in finger bowls containing filtered pond water at room temperature. For the investigation of the protein-bound amino acids the eggs in different stages of development were fixed separately in 80 per cent alcohol to precipitate the protein, and the two components of the egg, yolk and embryo, were carefully separated under the binocular microscope with fine needles. The yolk and embryo were then hydrolysed separately in 6N-HCl, and adopting the procedure of Giri *et al* (1952) the acid hydrolysates were prepared for chromatographic analysis. 150 eggs of each stage were used for the preparation of the acid hydrolysate. Two dimensional chromatographic analysis

on Whatman filter paper No. 1 was carried out using n-butanol, acetic acid and water mixed in the ratio of 4 : 1 : 1, v/v/v., as first solvent and 80 per cent buffered phenol (Block, 1952) as the second solvent. 0.5 per cent ninhydrin in 95 per cent acetone w/v was used as the colour developing reagent.

For the quantitative estimation of amino acids the following procedure was adopted. Each spot on the chromatogram was cut and eluted in 8.0 c.c. of a solution containing 7.2 c.c. of 80 per cent alcohol v/v, and 0.8 c.c. of 0.1 per cent copper sulphate solution w/v. The optical density of the solution was determined with Lumetron calorimeter model 401-A with 550 μ filter. The quantity was then calculated from the standard curve drawn for individual amino acids for known quantities of concentration.

Control chromatograms of known quantities of amino acids were run simultaneously under identical experimental conditions to locate the positions of individual amino acids and to serve as standards for quantitative analysis.

Results

The following amino acids were identified in the chromatograms of the acid hydrolysates of the yolk ; leucines phenylalanine, valine, methionine, proline, tyrosine, alanine, threonine, glycine, serine, glutamic acid, aspartic acid, histidine, arginine ornithine (probably an hydrolytic product of arginine) and cystine. During the development of the egg, the yolk showed six different patterns of distribution of protein-bound amino acids and the chief differences of these patterns are shown below.

Qualitative Changes :

Yolk :

TABLE I

Qualitative differences in the distribution of the protein-bound amino acids of the yolk.

Pattern	Methionine	Tyrosine	Histidine	Ornithine	Hours of Devpt.
I	+	+	+	–	Cleavage and Gastrulation
II	–	+	–	+	24, 72, 120
III	–	+	–	–	48, 144
IV	+	+	–	–	96
V	+	–	–	+	168
VI	–	–	–	–	192

In respect of other amino acids the different stages were all alike. Cystine was present in the yolk in all stages.

Embryo: The following amino acids were recognised in the chromatograms of the embryo : leucines, valine, proline, alanine, threonine, glycine, serine, glutamic acid, aspartic acid, arginine, asparagine and cystine.

During development the embryo showed only two different patterns of distribution of protein-bound amino acids in contrast to the six different patterns shown by the yolk. Till 72 hours of development leucines, valine, threonine, serine, aspartic acid, asparagine and cystine were lacking. Proline, on the other hand, was present only till 72 hours of development. From 72 hours of development onwards proline disappeared and the amino acids which were absent in the earlier stages, as mentioned above, appeared and continued to occur in all succeeding stages.

Phenylalanine, methionine, tyrosine, histidine and ornithine which were present in the yolk were not found in any of the developmental stages of the embryo.

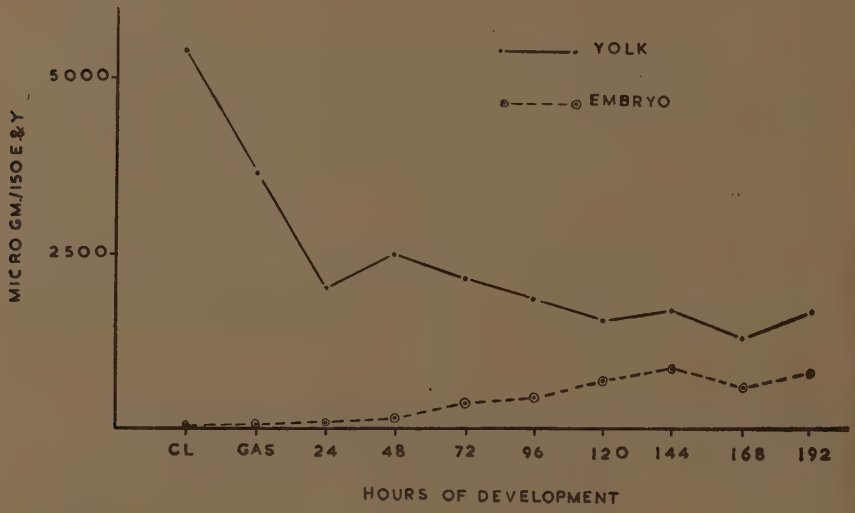
Quantitative Changes :

Yolk : The total concentration of the protein-bound amino acids of the yolk in the developing egg was greater than the embryo in all the stages, having a maximum during the cleavage stage. Thereafter there was an overall decline in the concentration of the amino acids until hatching. However at 48 and 120 hours of development all the amino acids excepting serine and asparagine showed a slight rise in comparison with the immediately preceding stage. Cystine in yolk showed a peak concentration during gastrulation.

Embryo: From 72 to 144 hours of development all the amino acids, excepting glycine and serine showed a gradual increase in concentration. At 168 hours of development there was a slight drop in concentration and in the subsequent stage of development all the amino acids showed an increase in concentration. Glycine and serine showed an increase in concentration during 120 hours of development and decrease afterwards.

From 120 hours of development to 192 hours the changes in the pattern of distribution of leucine, alanine, asparagine, aspartic acid and glutamic acid were similar in the yolk and embryo.

The ratio between the total amino acid content in the embryo and yolk shows an interesting variation in the different stages of development. Initially the ratio is greater *i.e.*, E : Y = 1 : 105. The ratio falls suddenly to 1 : 22 in the next two stages. Again there is a drop in the subsequent stages. During the last four stages, from 7th to 10th, the ratio is more or less constant.



Graph

Fig. 24. Protein-bound amino acids of the yolk and embryo during the different stages of development of *Oryzias melastigma*.

TABLE II

Amino acids during the development of *Oryzias melastigma*

No.	Amino acids	Hours of development: Clv.	Gast.	24	48	72	96	120	144	168	192
		Stages: 1	2	3	4	5	6	7	8	9	10
1	Leucines	—	—	—	—	16.87	16.85	22.50	61.87	28.12	44.01
2	Phenylalanine	—	—	—	—	—	—	—	—	—	—
3	Valine	—	—	—	—	9.83	19.55	19.65	49.12	26.20	48.12
4	Alanine	V.F.	6.00	8.40	12.60	21.00	16.80	25.20	46.20	34.40	50.96
5	Threonine	—	—	—	—	11.80	11.85	47.40	47.40	47.40	19.74
6	Glycine	V.F.	4.96	19.95	22.98	29.92	39.90	99.75	59.85	53.20	60.51
7	Serine	—	—	—	—	39.00	46.80	109.20	78.00	72.80	33.80
8	Glutamic acid	7.50	19.05	45.62	57.15	76.20	95.25	114.30	228.60	139.70	178.12
9	Aspartic acid	—	—	—	—	30.60	45.90	91.80	91.80	61.20	132.60
10	Arginine	V.F.	4.80	17.25	25.87	34.50	17.25	34.50	43.12	23.00	37.37
11	Asparagine	—	—	—	—	75.00	90.00	90.00	165.00	120.00	221.00
12	Cystine	—	—	—	—	24.00	24.00	24.00	24.00	16.00	20.80

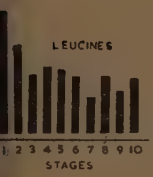
Quantitative changes in the protein-bound amino acids of the embryo. The quantity is expressed in micrograms/150 embryos.

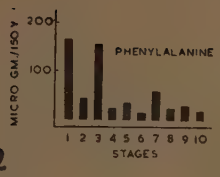
TABLE III

Amino acids during the development of *Oryzias melastigma*

No.	Amino acids	Hours of development									
		Clv.	Gast.	24	48	72	96	120	144	168	192
		Stages									
		1	2	3	4	5	6	7	8	9	10
1	Leucines	247.50	180.00	118.25	135.00	130.78	117.18	75.93	112.50	84.37	111.56
2	Phenylalanine	162.00	48.00	157.50	22.50	33.75	15.00	60.75	22.50	27.10	21.00
3	Valine	294.75	235.80	126.08	216.15	191.58	98.25	103.10	184.21	108.07	103.10
4	Alanine	336.00	224.00	142.30	168.00	138.60	133.00	90.00	126.00	84.00	117.60
5	Threonine	545.10	300.20	152.07	285.40	177.50	117.50	142.20	177.75	94.80	110.60
6	Glycine	299.25	199.50	93.10	169.57	134.60	99.75	74.58	85.40	69.80	93.10
7	Serine	436.80	332.80	182.00	151.80	245.70	221.00	163.80	109.20	140.00	191.10
8	Glutamic acid	838.20	609.60	322.26	476.25	414.33	349.25	271.46	369.09	257.17	333.37
9	Aspartic acid	826.20	428.40	214.20	351.90	275.40	255.00	206.55	210.37	153.00	214.20
10	Arginine	345.00	241.50	140.87	154.87	129.37	115.00	90.56	74.18	69.00	100.62
11	Asparagine	930.00	520.00	315.00	375.00	382.00	357.00	225.00	262.50	210.00	262.50
12	Cystine	192.00	256.00	56.00	48.00	108.00	40.00	36.00	30.00	48.00	56.00

Quantitative changes in the protein-bound amino acids of the Yolk. The quantity is expressed in micrograms/150 Yolk.





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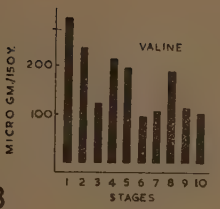

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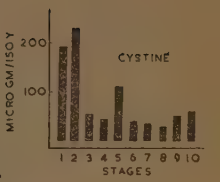

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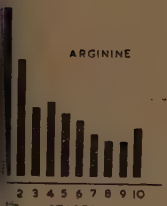

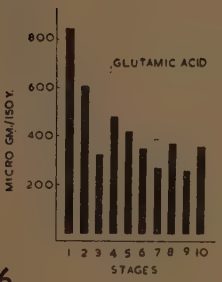

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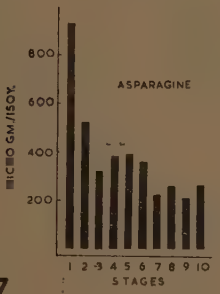

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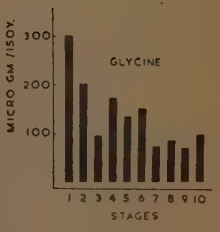

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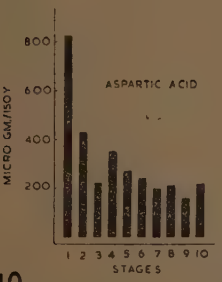

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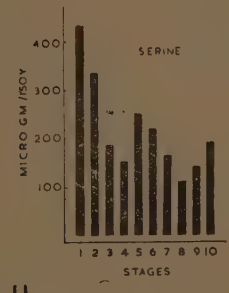

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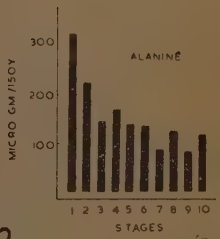
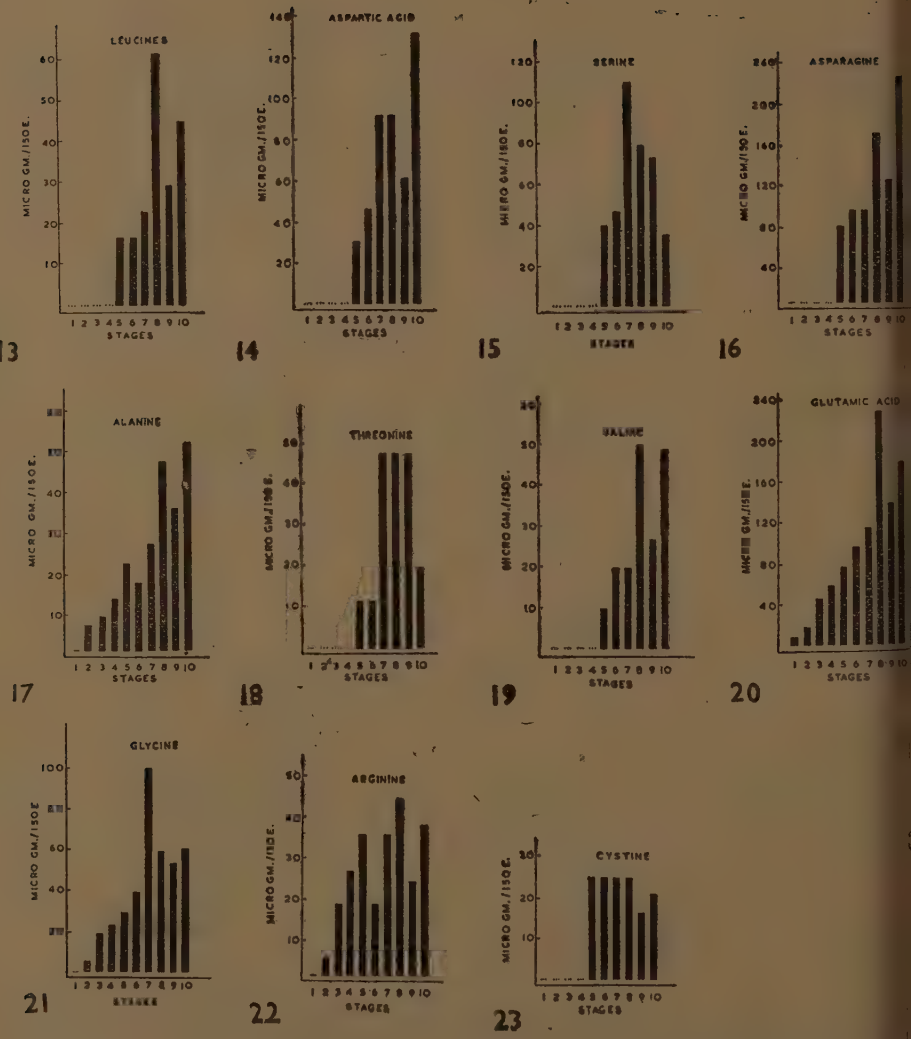


12

to 12. Relative changes in the concentration of the protein-bound amino acids of the yolk in the different stages of development of *Oryzias melastigma*.



Figs. 13 to 23. Relative changes in the concentration of the protein-bound amino acids of the embryo in the different stages of development of *Oryzias melastigma*.

————Traces of amino acids.

.....Complete absence of amino acids.

Discussion

The overall decline in the amino acid content of the yolk during the first 48 hours of development indicates that there is a rapid utilisation of the amino acids, of the yolk, by the embryo. As was explained earlier initially the ratio of total amino acid content in the yolk and embryo is 105 : 1. This drops down to 22 : 1 in the next two stages. After a further drop which means that there is still some utilisation of yolk amino acids the ratio of 2 : 1 is steady in stages 7 to 10. This shows that at first there is a very rapid utilisation and then less rapid and finally a steady and low utilisation.

The changes in the quantities of individual amino acids are not parallel in the yolk and embryo. Generally speaking it is the later stages of development that show a more or less parallel rise and fall in the content of individual amino acids in the yolk and embryo.

Summary

1. The protein-bound amino acids of yolk and embryo during the embryogenesis of *Oryzias melastigma* have been determined quantitatively by two dimensional chromatographic technique.

2. During the development of the egg the yolk shows six different patterns of distribution of protein-bound amino acids, whereas the embryo shows only two types of patterns.

3. In the early stages of development the quantities of individual amino-acids of the yolk and embryo fluctuates greatly but during the last stages of development most of the amino acids show parallel rise and fall.

4. The overall decline in the amino acid content of the yolk during the first 48 hours of development and the steady ratio maintained between the yolk and embryo during the last stages of development indicate that in the beginning there is very rapid utilisation of yolk amino acids by the embryo and towards the end a steady and low utilisation.

Acknowledgements

The author's thanks are due to Professor R. V. Seshaiya, Professor and Director, Marine Biological Station, Portonovo, for suggesting the problem, guidance and help, and to the Government of India, Ministry of Education for the award of a Senior Research Scholarship.

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LIPASE ACTIVITY IN THE ADIPOSE TISSUE OF VERTEBRATES

J. C. George and J. Eapen

Laboratories of Animal Physiology and Histochemistry, Department of Zoology, M. S. University of Baroda, Baroda

LIPASE was detected in the adipose tissue by earlier workers (Quagliariello and Scoz, 1932; Gomori, 1946). In recent years, however, some authors have expressed doubt as to the occurrence of a ' true lipase ' in the adipose tissue. Fawcett (1952) using a histochemical method (" Tween 40 " and " Tween 60 " as substrates) attempted the demonstration of lipase in the adipose tissue of the rat and reported that the enzyme under consideration may be an esterase. Korn and Quigley (1957) employing quantitative methods studied the same enzyme in the fowl adipose tissue and noted that it is a lipo-protein lipase. We attempted to establish the occurrence of a ' true lipase ', employing quantitative as well as histochemical methods, in the adipose tissue of different animals (1958a, b ; 1959a, b, c) and the evidences we have on hand indicate the presence of a ' true lipase ' in this tissue. We (1958b) have demonstrated histochemically the presence of lipase in the adipose tissue of the fowl and the rosy pastor. In the present paper we report the results of a quantitative study of lipase activity in the frog (*Rana tigrina*), lizard (*Calotes versicolor*), fowl (*Gallus domesticus*) and rosy pastor (*Pastor roseus*) adipose tissue.

Materials and Methods

In all cases the visceral adipose tissue was used. The adipose tissue covering the coils of the intestine in the fowl and the rosy pastor and the adipose tissue found in association with the reproductive organs in the frog and the lizard were carefully dissected out after decapitation of the animal, separately minced and defatted in two changes of ethyl ether at room temperature (30°C) for 2 hours. The defatted tissue was dried in a vacuum desiccator at room temperature. An extract of each was made in cold distilled water by grinding with clean sand by means of a glass rod in a test tube for 1 hour in cold (4°C), centrifuged at approximately 2500 r. p. m. for 5 min. and the resulting supernatant was used as the enzyme material.

The method of assay employed was a manometric method adopted from Martin and Peers (1953), with a bicarbonate carbon dioxide buffer system of pH 7.4 at 37°C using the Warburg apparatus. The reaction flask contained 1.5 ml of 0.025 M bicarbonate solution and 1 ml enzyme solution in the main chamber and 0.5 ml 4% (v/v) tributyrin in 0.0148 M bicarbonate solution (emulsified by shaking with a drop of " Tween 80 ") in the side arm thus making up a total volume of 3 ml. The procedure was the same as followed by us in an earlier study (1959 b). Protein in the enzyme solution was estimated by the micro-Kjeldahl steam distillation method for total proteins (Hawk *et al*, 1954). The enzyme activity is expressed as μl CO_2/ mg protein/ hour.

Results and Discussion

Table 1 presents the lipase value in the adipose tissue of the four vertebrates studied as well as the values obtained by us in our earlier studies for the same enzyme in the pigeon and bat adipose tissue, for comparison.

TABLE I

Animal	Lipase activity : μl CO_2/mg protein/hour
frog	16·43
lizard	275.85
fowl	21.02
rosy pastor	254.40
pigeon*	246.40
bat**	
(yellow)	37.85
(brown)	30.45

* George and Eapen, 1958 a. ** George and Eapen, 1959 b.

The lipase activity in the two birds studied gave contrasting results, rosy pastor showing very high lipase activity and the fowl comparatively low. This is in conformity with the results of our histochemical study on the adipose tissue of these two birds (1958 b). We suggested a possible explanation for the higher lipase concentration in the rosy pastor adipose tissue in that it is an indication of higher metabolic activity especially involving lipid metabolism. This is noteworthy due to the fact that the rosy pastor is a migrant and the birds used in the present study were pro-

cured few days before they were to start on their migration to Europe. For the synthesis of the large amount of fat (mainly triglycerides) accumulated in the adipose tissue during the premigratory period, considerable amount of lipase should be deemed essential. In the fowl also at times adipose tissue is found to contain large quantities of fat, but the lipase activity when calculated on the basis of the protein concentration of the enzyme solution, remains low. This may be because, all the fat that is found in the adipose tissue of the fowl may not be synthesized there but transported from other sites of synthesis and deposited in the adipose tissue. This view is apparently supported by the findings of Bumgardner (1957) who investigated the origin and mobilization of the fatty acids of the fat bodies of avian embryos using tracer techniques. According to this author a large majority of the synthesized fat found in the fat body of the chick embryo has its origin in other tissues. But this however, does not disprove the fact that the synthesis of fat takes place in the adipose tissue itself. If so, the ability to synthesize fat (triglycerides) by the adipose tissue, may be different in different animals and the concentration of lipase may be regarded as an index of the varying capacities for fat synthesis. The amount of lipids present in the adipose tissue of the frog and the lizard is reported to be almost the same (Shah, 1952). But the lipase activity in this lizard adipose tissue is comparatively very high. The condition in these two animals parallels that of the fowl and rosy pastor respectively. We also observed that there is a seasonal variation in the lipase concentration of the frog adipose tissue. The value of 16.43 μl CO_2/ mg protein/ hour (Table 1) obtained during mid-summer (May) rose to 21.60 μl CO_2 / mg protein/ hour during the rains (July). These observations lead to show that irrespective of the fact that an animal is aquatic or terrestrial the lipase activity in the adipose tissue is an index of the extent of fat synthesis or breakdown in this tissue. It also shows that lipase activity is less in the adipose tissue of those animals in which the fat is gradually built and gradually used up, but on the other hand it is very high in those in which fat is built up rapidly for a large scale utilization in a short period as is the case with migratory birds before and during migratory flights. The high figure in the lizard which may be true for other reptiles as well, is perhaps due to the fact that they are constantly threatened with starvation during some seasons and also they lay large yolked eggs. Moreover for a terrestrial reptile more fat stored means more water conserved and more fat utilized

means more metabolic water made available to meet the acute conditions of draught. Further it should be stated that fowl adipose tissue was reported to be containing only lipo-protein lipase and no lipase (Korn and Quigley, 1957). But our studies have revealed the occurrence of appreciable quantities of ' true lipase ' in the adipose tissue of the fowl.

Summary

Lipase activity in the visceral adipose tissue of the frog, lizard, fowl and rosy pastor were determined by manometric method using the Warburg apparatus. The concentration of the enzyme is comparatively high in the lizard and the rosy pastor while in the frog and the fowl it is low. The significance of the observations is discussed.

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OBITUARY

The Late Professor Lewis Victor Heilbrunn

We have received with profound sorrow the news of the untimely and tragic death of Professor L. V. Heilbrunn in a car accident. In him the scientific world has lost a great biologist who has been a source of inspiration not only to the generations of students who passed through his hands but also to others who happened to read his works.

Dr. Heilbrunn was born on the 24th of January 1892. Graduated at the University of Cornell in 1911 and took the Doctorate Degree in Zoology in 1914 at the University of Chicago. Thereafter he served on the staff of the Universities of Chicago and Michigan. He was appointed Associate Professor of Zoology at the University of Pennsylvania in 1929 and in 1943 was raised to the post of professor which he held until his death. He was an International authority on protoplasm and was Joint Editor of *Protoplasma Monographien* (1928-35) and *Protoplasmatologia*. He was also a member of the editorial board of some of the leading biological journals and of several learned bodies. He was a trustee of the Marine Biological Laboratory at Woods Hole, Vice-president of the American Society of Zoologists (1932) and President of the Society of General Physiologists (1946). As a general physiologist his works on the physiology of cell division, protoplasmic viscosity and protoplasmic clotting are great contributions to modern biology. As an author his "Outlines of General Physiology" still remains a classic in which the reader is not only confronted with known facts but also discussions initiated in a masterly fashion on the uncertainties of knowledge, thereby stimulating him towards a greater search for the unknown. His other books are "Colloidal Chemistry of Protoplasm" (1928) and "The Dynamics of Living Protoplasm" (1956). As a teacher he was superb. The very fact that some of the leading biologists of America and other countries were his former students, speaks volumes for the qualities of the man as a teacher.

We extend our sincere sympathies to his bereaved wife Ellen and daughter Constance.

J. C. George

which published (only recognised abbreviations of journals, underline) Volume number (double underline) and page number. *e.g.*

Haines, R. W. 1934 The homologies of the flexor and adductor muscle of the thigh. *J. Morph.*, **56**, 21.

Titles of papers may be omitted altogether. Books should be listed as follows : Author's last name followed by initials, year of publication, title, publishers and place of publication. *e.g.*

Heilbrunn, L. V. 1952 An Outline of General Physiology. W. B. Saunders Company, Philadelphia.

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